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Hepatic-portal oleic acid inhibits feeding more potently than hepatic-portal caprylic acid in rats

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Abstract

In several human and animal studies, medium-chain triglycerides decreased food intake more than did long-chain triglycerides. It is possible that faster uptake and metabolism of medium-chain fatty acids in the liver is responsible for this difference. To test this hypothesis we compared the feeding effects of hepatic portal vein (HPV) infusion of the medium-chain fatty acid caprylic acid (CA) with those of the long-chain fatty acid oleic acid (OA). Contrary to our expectation, six-h HPV infusion of 14 µg/min (50 nmol/min) OA robustly inhibited feeding, whereas infusion of 22 or 220 µg/min (150 and 1500 nmol/min) CA failed to have any effect on feeding. Only a much larger dose of CA, 1100 µg/min (7500 nmol/min) inhibited feeding similarly to 14 µg/min OA. The increased feeding-inhibitory potency of OA did not appear to be due to differences in stimulation of hepatic fatty acid oxidation because equimolar (50 nmol/min) doses of OA (14 µg/min) and CA (7 µg/min) did not differentially affect post-infusion levels of β-hydroxybutyrate. Stress, inflammation, acute hepatotoxicity or oxidative stress also do not appear to account for the increased feeding-inhibitory potency of HPV OA because plasma concentrations of the stress hormones corticosterone and epinephrine, the pro-inflammatory cytokines interleukin-6 and tumor necrosis factor-α, the liver enzymes γ-glutamyl transferase and alanine aminotransferase and as well as hepatic levels of malondialdehyde and glutathione were all similar after HPV infusion of saline or of 50 nmol/min OA or CA.

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Keywords: Satiety; Liver; Fatty acid oxidation; Long-chain fatty acids; Medium-chain fatty acids

1. Introduction

Several studies in both animals [5,8,12,17] and humans [26,31,32] suggest that medium-chain triglycerides (MCT) enhance satiety and decrease food intake more than long-chain triglycerides (LCT). The reason for this difference is unknown, but the differences in post-absorptive handling of MCT and LCT may be important. First, medium-chain fatty acids (MCFA) derived from digestion of MCT are absorbed directly into the hepatic portal vein (HPV), whereas long-chain fatty acids (LCFA) from LCT are packaged into chylomicrons and absorbed into the lymphatic system [23], thus bypassing the liver. Second,

the transport of MCFA into the mitochondria does not require carnitine palmitoyltransferase-1, which is the rate-limiting step of mitochondrial transport of LCFA [33]. Both of these effects would tend to favor hepatic oxidation of MCFA compared to LCFA. Consistent with this, oxidation of MCFA was reported to increase ketogenesis — an indicator of hepatic fatty acid oxidation (FAO) — more than did the oxidation of LCFA in humans [32]. Because hepatic FAO is thought to inhibit eating, (for review see Refs. [15,18]) the more pronounced effect of MCFA on hepatic FAO could explain the apparently superior satiating potency of MCT. This reasoning would also suggest that MCFA would be more satiating than LCFA. Cox et al. [7] and Meyer et al. [20], however, reported the opposite result: Intra-intestinal infusions of the MCFA caprylic acid (CA) reduced food intake less, not more, than isoenergetic intra-intestinal infusion of the LCFA oleic acid (OA). One potential explanation for this is that OA elicited more potent pre-absorptive feeding-inhibitory

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signals than CA [7], and that this difference overshadowed the stronger post-absorptive feeding-inhibitory signal arising from hepatic oxidation of CA. We tested this hypothesis in the current experiments by comparing the effects of HPV infusions of OA and CA on food intake.

2. Methods

2.1. Animals

Male rats weighing 400–590 g during tests were bred locally in SPF conditions using Sprague–Dawley founders from Charles River Germany. For tests, rats were individually housed in wire-mesh cages with ad libitum access to water and ground rat chow (No. 3433, Provimi Kliba, Kaiseraugst, Switzerland) with a metabolizable energy content of 12.4 kJ/g. The colony room was temperature-controlled ($22 \pm 2^\circ\text{C}$) with a reversed 12:12-h light–dark cycle. Rats were adapted to these housing conditions and to daily handling and weighing for at least 8 days before surgery. All protocols for these experiments were approved by the Veterinary Office of the Canton of Zurich.

2.2. Surgery

Rats were anesthetized by intraperitoneal injection (1.25 ml/kg) of 100 mg/kg ketamine (Ketasol-100, Dr. E. Gräub AG, Bern, Switzerland), 5 mg/kg xylazine (Rompun, Bayer, Leverkusen, Germany), and 0.05 mg/kg acepromazine (Pre-quillan, Arovet AG, Zollikon, Switzerland). One or two intravascular catheters were implanted using a sterile technique, as described below. For postoperative analgesia rats received subcutaneously 5 mg/kg/d carprofen (Rimadyl, Dr. E. Gräub AG) for the following 2 days.

2.2.1. HPV catheters

Catheters and headsets were custom-made from silicon tubing (ID 0.51 mm, OD 0.94 mm, length 27 cm; Ulrich Swiss, St. Gallen, Switzerland), a 20-gauge Vacutainer cannula (Becton–Dickenson, Basel, Switzerland), and polypropylene surgical mesh (2.5×2.5 cm, Bard mesh, Oberrieden, Switzerland), implanted in the ileocolic vein and advanced into the HPV, exteriorized in the interscapular area, and fitted with caps as previously described [27]. Silk suture (Silkam, 3/0, Braun, Melsungen, Germany) and glue (Histoacryl, Braun) were used to fix the catheter to the ileocolic vein, and resorbable suture (3-0 Vicryl, Ethicon GmbH, Norderstedt, Germany) was used to close the skin and muscle. During the first postoperative week the catheters were flushed daily with 0.3 ml 0.9% sterile saline and filled with 80 μl heparinized saline (100 I.U./ml heparin, Braun), thereafter every second day.

2.2.2. Jugular vein catheters

Silicon (ID 0.51 mm, OD 0.94 mm, length 10.5 cm) catheters were implanted into the jugular vein and advanced to the right atrium using the original method of Steffens [24], modified as previously described [10], exteriorized 1 cm rostral to the HPV catheter, and fitted with caps. Catheters were flushed every day

with 0.2 ml 0.9% saline and then filled with 70–100 μl PVP-heparin (Polyvinylpyrrolidone, Sigma, Buchs, Switzerland).

2.3. Test procedure

About 1 week after surgery, rats were housed individually in open-topped plexiglass cages ($37 \times 21 \times 41$ cm) with stainless steel grid floors. Rats had ad libitum access to water, in sipper tubes hung outside the cage, and ground chow, which was in food cups placed under a 39 cm^2 hole at the end of a niche in the cage wall that was 4.6 cm wide, 7 cm deep, and 29.5 cm high, with the base 5 cm above the cage floor. The cups were mounted on electronic balances (Mettler PM 3000, Greifensee, Switzerland) that were interfaced with a computer (Olivetti M 300, Nürnberg, Germany) in an adjacent room. A custom-designed program (VZM; Krügel, Munich, Germany) recorded the weights of the food cups every 30 s, enabling continuous measurement of spontaneous feeding patterns. In addition, a video system (Enhanced Observation Kit VSS 2285/OOT, Philips, Zurich, Switzerland) permitted continuous observation of the rats.

Each day the HPV catheter was attached to a syringe pump (model A99; Razel, Stamford, CT, USA) mounted above the cage via a segment of Tygon tubing (0.76 mm ID, 1.22 mm OD; Portex, Hythe, Kent; UK) sheathed with a stainless steel spring and connected to a swivel joint fixed ~ 45 cm above the cage floor, which allowed the rat to move freely. The infusion pumps were remotely controlled from the adjacent room. Six-hour infusions (22 $\mu\text{l}/\text{min}$) began 1 h prior to dark onset. At the end of the infusions the catheter was detached and flushed, and the headset was capped. Rats were adapted to this procedure several times before tests.

The patency of the hepatic portal vein catheters was checked at sacrifice of the rats. We verified whether the tip of the catheter

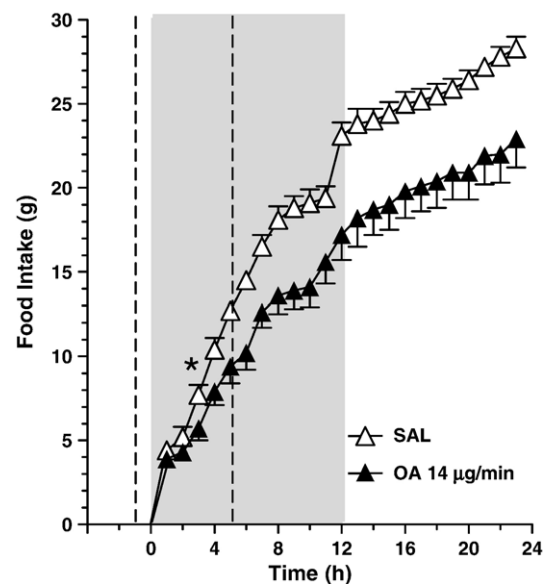


Fig. 1. Effects of HPV infusion of 14 $\mu\text{g}/\text{min}$ OA and equimolar saline on cumulative food intake in 2 h-food deprived rats re-fed at dark onset. Infusions began 1 h prior to dark onset and ended 6 h later (dotted lines). Gray background indicates dark phase. Data are means \pm SEM intakes of 13 rats tested using a crossover design. SAL, saline; OA, oleic acid. *Significantly different from SAL at 3 h and all subsequent time points; Tukey test after significant ANOVA, $P < 0.05$.

Table 1
Effects of hepatic portal vein caprylic acid (CA) and oleic acid (OA) infusions on nocturnal feeding

Dose of test solutions	12 h FI (g)	Number of meals	Meal size (g) average	Duration (min) average	IMI (min) average
220 µg/min (n=12)					
SAL	22.6±0.8	8.3±0.4	2.8±0.1	13.9±1.2	80.0±1.2
CA	22.5±0.7	8.5±0.5	2.7±0.1	14.4±1.1	74.7±5.0
1100 µg/min (n=12)					
SAL	19.3±0.9	8.3±0.6	2.4±0.1	11.4±1.0	79.0±5.7
CA	14.7±1.3*	6.8±0.7	2.2±0.1	12.4±1.3	95.5±8.8
14 µg/min (n=13)					
SAL	23.1±0.8	7.2±0.5	3.2±0.2	10.7±0.7	90.8±7.2
OA	17.2±1.5**	5.8±0.5*	3.0±0.2	11.4±1.0	110.2±10.9

Data are means±SEM; FI, food intake; IMI, inter meal interval; SAL, saline.

* $P<0.05$; ** $P<0.01$.

was still in the portal vein and whether the catheter was still open and did not leak. Only rats that met these criteria were included in the data analysis.

2.4. Experimental designs

2.4.1. Experiment 1: effect of HPV OA infusion on food intake

The effects of HPV infusions of OA (sodium oleate, Sigma; OA equivalents below) on feeding were tested in 13 rats with HPV catheters. Rats were food deprived for 2 h, and food was returned at the beginning of the dark phase, 1 h after the infusion started. Six-hour infusions of OA (50 nmol/min, 14 µg/min), or equimolar, equal pH saline were tested using a crossover design, with at least 2 days of ad libitum feeding between trials. Sterile infusion solutions 2.3 mmol/l sodium oleate (corresponding to 0.65 g/l OA) were prepared daily by heating 15 min at 56 °C, adjusting the pH to 7.4, and filtering (Nalgene 0.2 µm, Nalge Nunc International, Rochester, NY, USA).

2.4.2. Experiment 2: Effects of HPV CA infusion on food intake and plasma metabolites

The effects of HPV infusions of 3 different doses of CA (sodium caprylate, Sigma; CA equivalents below) on feeding and plasma metabolites were tested in a new group of 12 rats with HPV and jugular vein catheters. As in Experiment 1, rats were food deprived for 2 h, and food was returned at the beginning of the dark phase, 1 h after the infusion started. Six-hour infusions of CA (150, 1500 and 7500 nmol/min; 22, 220 and 1100 µg/min, respectively) or equimolar, equal pH saline were tested using a crossover design, with at least 2 days of ad libitum feeding between trials. Sterile infusion solutions of 7 mmol/l (1.0 g/l), 70 mmol/l (10.1 g/l) and 350 mmol/l (50.5 g/l) CA were prepared daily by heating 5 min at 45 °C, adjusting the pH to 7.4, and filtering (Nalgene 0.2 µm, Nalge Nunc International, Rochester, NY, USA).

At the end of the infusion, the jugular catheters were attached and a 900-µl blood sample was taken. An equal volume of saline was then infused, and the catheter was detached and refilled with PVP-heparin. Blood was mixed with NaF (3 mg/ml whole blood), stored on ice during the experiment, and centrifuged for 8 min at 8000 rpm at 4 °C immediately after-

wards. Plasma was aliquoted and stored at -20 °C. Glucose, free fatty acids (FFA), and β-hydroxybutyrate (BHB) were analyzed as previously described [16].

2.4.3. Experiment 3: effects of HPV OA and CA infusions on plasma metabolites, stress hormones, liver enzymes and cytokines

Blood samples were taken in a situation that was similar to the feeding tests, i.e., during a crossover test of OA and CA (50 nmol/min), with at least 2 days between consecutive experiments, in food-deprived rats ($n=8$ new rats, with HPV and jugular vein catheters). Two hours before lights out, food was removed and the

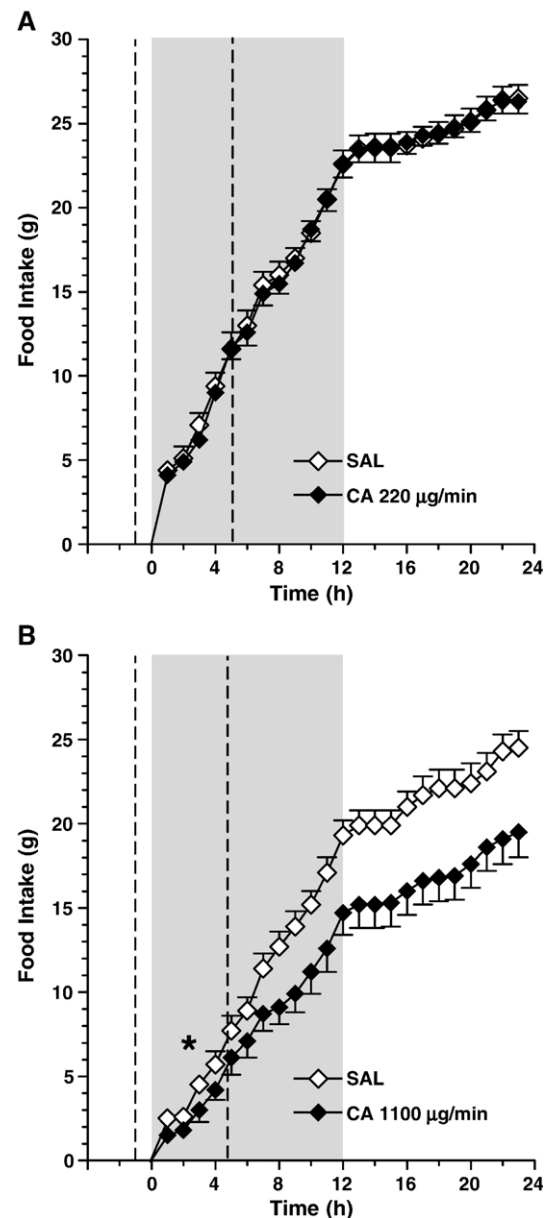


Fig. 2. Effects of HPV infusions of 220 µg/min (A) and 1100 µg/min (B) CA and equimolar saline on cumulative food intake in 2 h-food deprived rats re-fed at dark onset. Infusions began 1 h prior to dark onset and ended 6 h later (dotted lines). Gray background indicates dark phase. Data are means±SEM intakes of 12 rats tested using a crossover design. SAL, saline; CA, caprylic acid. *Significantly different from SAL at 3 h and all subsequent time points; Tukey test after significant ANOVA, $P<0.05$.

jugular vein catheters were attached. About 45 min later a basal 700- μ l blood sample was withdrawn and 700- μ l saline was injected. The jugular vein catheters were then detached and filled with PVP-heparin. Thereafter, the HPV catheters were attached and 75 min before lights out, a 3.0–3.2 g chow pellet was offered, and 15 min later the remaining food was removed, intake was verified to be at least 2.8 g, and 6 h infusions of OA or CA began. Rats had no access to food during infusions. At the end of the infusion, the jugular catheters were attached and 30 min later a 700- μ l blood sample was taken. An equal volume of saline was infused afterwards, and the jugular vein catheter was then detached and refilled with PVP-heparin. Blood was mixed with EDTA (1.8 mg/ml whole blood), stored on ice during the experiment, and then centrifuged for 8 min at 8000 rpm at 4 °C. Plasma aliquots were stored at –70 °C. Corticosterone was analyzed by RIA (Linco Research, St. Charles, MI, USA), epinephrine was measured by HPLC with electrochemical detection [22], the pro-inflammatory cytokines tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6) were measured by immunoassay (Linco Research) in a Luminex¹⁰⁰ (Linco Research), and the other assays were performed as previously described [16].

2.4.4. Experiment 4: effects of HPV OA and CA infusions on hepatic malondialdehyde (MDA) and glutathione (GSH) levels

Rats ($n=13$, previously used in experiment 3) were divided in three groups with 3 or 4 rats per group. Two hours before lights out food was removed and 1 h later HPV catheters were attached. A 3.0–3.2 g chow pellet was offered, and 15 min later the remaining food was removed, intake was verified to be at least 2.8 g, and 6 h infusions of OA (50 nmol/min, 14 μ g/min), CA (7500 nmol/min, 1100 μ g/min) or SAL (equimolar to CA) began. Rats had no access to food during infusions. At the end of the infusion, the HPV

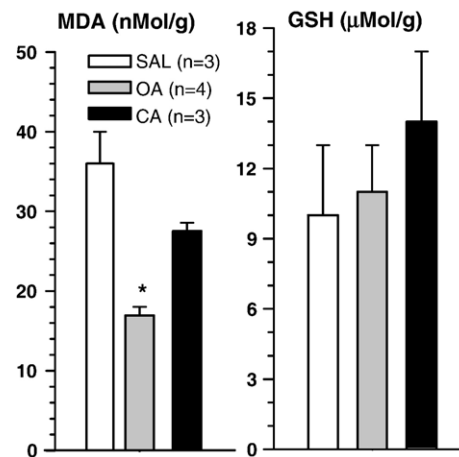


Fig. 3. Effects of HPV infusion of 14 μ g/min OA, 1100 μ g/min CA and saline (equimolar to CA) on hepatic lipid peroxidation as determined by malondialdehyde (MDA) and glutathione (GSH) levels at the end of the infusion. Data are means \pm SEM. SAL, saline; OA, oleic acid; CA, caprylic acid. *Significantly different from SAL, Kruskal–Wallis test followed by Mann–Whitney U -test, $P<0.05$.

catheters were detached and rats were anesthetized with ether. A liver sample was taken and homogenized in 600 μ l/100 mg tissue of PBS (pH 7.4) containing 1% CHAPS (Pierce, Rockford, IL, USA). To analyze MDA, the homogenate containing 5 mM butylated hydroxytoluene in 20 mM Tris was centrifuged to remove cellular debris, purified using the methanesulfonic acid solvent extraction procedure, and absorbance was measured at 586 nm [1]. GSH levels were measured using a method based on the formation of a chromophoric product resulting from the reaction of 5,5'-dithio-bis-(2-nitrobenzoic acid) (DTNB, Sigma) and GSH (Calbiochem, San Diego, CA, USA) [29].

2.5. Data collection and analysis

Meals were defined as weight changes ≥ 0.3 g, lasting ≥ 1 min, and separated by ≥ 15 min from other weight changes [27]. Meal duration was the time from the first to the last weight change in a single meal, and the inter-meal interval (IMI) was the time from the last weight change in a meal to the first change in the next meal. Data are expressed as means \pm SEM. Cumulative food intakes (experiments 1 and 2), plasma metabolites and stress hormones (experiment 3) were analyzed by repeated-measures analysis of variance (ANOVA) with infusion and time as factors, and post hoc comparisons between means were done with Tukey's HSD test. Meal pattern (experiments 1 and 2) and the other plasma parameters (experiments 2 and 3) were analyzed with paired t -tests. In Experiment 4, the data did not meet the requirements for ANOVA and were analyzed by a Kruskal–Wallis test followed by Mann–Whitney U -test. Minimum level of significance was $P<0.05$.

3. Results

3.1. Experiment 1: effect of HPV OA infusion on food intake

Six h HPV infusions of 50 nmol/min (14 μ g/min) OA reduced cumulative food intake from 3 h after the start of infusions until

Table 2

Plasma metabolites, stress hormones, liver enzymes and cytokines before (basal) and after hepatic portal vein infusion (6 h) of caprylic acid (CA) and oleic acid (OA)

	CA (7.2 μ g/min)		OA (14 μ g/min)	
	50 nmol/min		50 nmol/min	
	Basal	6 h	Basal	6 h
Metabolites				
Glu (mmol/l)*	8.1 \pm 0.1	6.7 \pm 0.2	8.3 \pm 0.2	6.6 \pm 0.2
FFA (μ mol/l)*	198 \pm 22	662 \pm 55	214 \pm 18	664 \pm 78
BHB (μ mol/l)*	43 \pm 9	448 \pm 48	44 \pm 13	485 \pm 56
Hormones				
Cort (μ g/l)*	73 \pm 6	107 \pm 11	64 \pm 9	115 \pm 15
Epi (pg/ml)*	23 \pm 10	61 \pm 10	20 \pm 7	48 \pm 10
Liver enzymes				
ALT (U/l)		40 \pm 3		42 \pm 3
γ -GT (U/l)		1.1 \pm 0.2		0.9 \pm 0.1
Cytokines				
TNF- α (pg/ml)		14 \pm 6		14 \pm 9
IL-6 (pg/ml)		861 \pm 398		1072 \pm 415

Glu, glucose; FFA, free fatty acids; BHB, β -hydroxybutyrate; Cort, corticosterone; Epi, epinephrine; Alt, alanine amino-transferase; γ -GT, γ -glutamyl transferase; TNF- α , tumor necrosis factor- α ; IL-6, interleukin-6.

Data are means \pm SEM, $n=8$.

*6-h value significantly different from basal value, $P<0.05$.

the end of the test (Fig. 1; main effect of drug, $F(1, 276)=16.4$; $P<0.01$; interaction effect of drug and time, $F(23, 276)=3.5$; $P<0.0001$). This reduction was due mainly to a significant decrease in the number of meals during the dark phase without a significant change in nocturnal meal size (Table 1).

3.2. Experiment 2: effects of HPV CA infusion on food intake and plasma metabolites

Six h HPV infusions of 150 (22 $\mu\text{g}/\text{min}$) or 1500 nmol/min (220 $\mu\text{g}/\text{min}$) CA did not affect either cumulative food intake (Fig. 2A) or meal patterns (Table 1; data for lower CA dose not shown). HPV infusion of 7500 nmol/min (1100 $\mu\text{g}/\text{min}$) CA significantly reduced cumulative food intake (Fig. 2B) from 3 h after the start of infusions until the end of the test (main effect of drug, $F(1, 242)=10.1$; $P<0.01$; interaction effect of drug and time, $F(22, 242)=9.4$; $P<0.0001$). No reliable changes in meal size or meal number were detected (Table 1).

HPV CA infusion did not significantly affect the plasma concentration of any of the parameters examined. Following infusion of 1100 $\mu\text{g}/\text{min}$ CA, plasma glucose levels were 7.7 ± 0.2 mmol/l (SAL) and 7.6 ± 0.2 mmol/l (CA), $P>0.10$, BHB levels were 30 ± 7 $\mu\text{mol}/\text{l}$ and 38 ± 8 $\mu\text{mol}/\text{l}$, $P>0.10$, and FFA levels were 0.13 ± 0.01 mmol/l and 0.30 ± 0.09 mmol/l, $t(9)=1.873$; $P=0.09$.

3.3. Experiment 3: effects of equimolar HPV OA and CA infusions on plasma metabolites, stress hormones, liver enzymes and cytokines

The 6 h values of all the metabolites (glucose, FFA and BHB) and stress hormones (corticosterone and epinephrine) examined differed significantly from the basal values (Table 2), but there were no significant main effects of OA and CA infusions and no significant time-infusion interactions. Post-infusion plasma levels of the pro-inflammatory cytokines TNF- α and IL-6 and the liver enzymes alanine aminotransferase (ALT) and γ -glutamyl transferase (γ -GT) also did not differ significantly between the two groups (Table 2).

3.4. Experiment 4: effects of HPV OA and CA infusions on hepatic MDA and GSH levels

Neither OA nor CA infusion increased the production of reactive oxygen species (ROS) as assessed by hepatic MDA or GSH levels (Fig. 3). Indeed, MDA levels were significantly reduced after OA infusion.

4. Discussion

Extensive evidence indicates that hepatic FAO can control food intake [11,15,18]. This study was designed to test the hypothesis that, due to the more rapid hepatic oxidation of MCFA than LCFA, HPV infusion of the MCFA CA would inhibit feeding more potently than HPV infusion of comparable amounts of the LCFA OA. The results disconfirmed this hypothesis. Under our conditions, HPV infusion of 50 nmol/min (14 $\mu\text{g}/\text{min}$) OA produced a robust inhibition of feeding, whereas HPV infusion of

150 and 1500 nmol/min (22 and 220 $\mu\text{g}/\text{min}$) CA failed to have any effect on feeding. Feeding-inhibitory effects similar to those of 50 nmol/min (14 $\mu\text{g}/\text{min}$) OA were observed after HPV infusion of 7500 nmol/min (1100 $\mu\text{g}/\text{min}$) CA, i.e., 150 times the molar dose and 80-times the weight dose. Why HPV OA was so much more potent than HPV CA is not clear. Grekin et al. [13] calculated that HPV infusion of 250 nmol/min OA increased hepatic OA delivery by only 12% and total fatty acid delivery by 2%. Thus, the 5-times smaller OA dose we used seems very unlikely to increase hepatic FAO more than the larger dose of CA. Contrary to our expectation, plasma BHB levels were not significantly increased after the larger dose of CA in Experiment 2. At first glance this suggests that even this CA dose did not increase hepatic FAO. Yet, plasma BHB is only an indirect measure of hepatic FAO, and the failure to detect an increase in plasma BHB levels is probably due to the fact that the rats had access to chow throughout this test, which rapidly decreases ketogenesis and, hence, the ratio of BHB to total fatty acids in the plasma [34]. Indeed, we recently demonstrated that HPV CA attenuated the drop in plasma BHB levels when rats were offered a 4-g test meal after 18 h of food deprivation [14], and in Experiment 3, when the rats were given only a 3 g test meal before the onset of infusions, plasma BHB at the end of the infusions was similarly increased by 7 $\mu\text{g}/\text{min}$ CA and 14 $\mu\text{g}/\text{min}$ OA. All in all, our data do not support the hypothesis that the rate of hepatic FAO determines the differential feeding-inhibitory potencies of circulating CA and OA.

Our feeding data are qualitatively similar to reports that intra-intestinal infusions of OA inhibited feeding more than similar doses of CA [7,20,35]. Furthermore, as in our study, intra-intestinal infusion of OA decreased food intake by reducing the number of meals without affecting meal size [7]. As a substantial part of the inhibition of feeding produced by intra-intestinal fat infusions is based on pre-absorptive signaling mechanisms [2,4,25,30,36], however, the relevance of these data to the effects of post-absorptive nutrient infusions is uncertain.

The different feeding-inhibitory potencies of OA and CA may be related to differential effects on hepatic vagal afferent activity. Vagal afferent signaling apparently mediates much of the control of food intake by lipids in the small intestine [6,7,35] as well as by hepatic lipid metabolism [18]. Vagal receptors with different sensitivity to LCFA and MCFA have been identified in the small intestine [19], and HPV infusion of the LCFA linoleic acid increased hepatic vagal afferent activity more than the same amount of CA [28].

Finally, although administration of large doses of LCFA and MCFA can have nonspecific or toxic effects that could inhibit feeding differentially, such effects did not seem to occur here. For example, intra-duodenal infusions of 7.2 or 14.4 mg/min sodium OA for 10 min, but not equicaloric doses of sodium CA, increased intestinal lactate dehydrogenase activity, which is a marker for cell death, and produced a conditioned taste aversion [21]. Similarly, HPV infusion of 42 $\mu\text{g}/\text{min}$ OA for 24 h markedly increased plasma concentrations of the stress hormone corticosterone [3] and the liver enzyme ALT, which is a marker for hepatocyte damage. In contrast, in our study the effects of 6 h HPV infusion of 14 $\mu\text{g}/\text{min}$ OA on plasma corticosterone and

epinephrine did not differ from HPV infusion of equimolar CA. The significantly higher 6 h values in comparison to the basal values might be related to the fasting because rats had no access to food during the infusion. Furthermore, HPV OA infusion did not increase plasma levels of the liver enzymes ALT and γ -GT or of the pro-inflammatory cytokines TNF α and IL-6 in comparison to equimolar HPV CA. Finally, neither HPV OA nor CA induced oxidative stress in liver cells as assessed by hepatic concentrations of MDA or GSH. Indeed, HPV OA significantly reduced hepatic MDA levels, perhaps because moderate amounts of LCFA apparently can enhance ROS degradation [9].

In conclusion, the present data provide evidence that HPV infusions of OA in doses that fail to produce overt signs of toxicity inhibit feeding markedly more than HPV infusions of CA. This did not appear to be due to differential effects on hepatic FAO. The mechanisms involved remain unclear. Given the importance of dietary fat in the control of food intake and body weight, the apparent paradox between one body of research suggesting that MCT ingestion reduces food intake more than LCT and another body of research indicating that intra-intestinal and HPV infusion of LCFA reduces food intake more than infusion of MCFA deserves further research.

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References

- [1] Arsenijevic D, de Bilbao F, Giannakopoulos P, Girardier L, Samec S, Richard D. A role for interferon- γ in the hypermetabolic response to murine toxoplasmosis. *Eur Cytokine Netw* 2001;12:518–27.
- [2] Beglinger C, Degen L. Fat in the intestine as a regulator of appetite-role of CCK. *Physiol Behav* 2004;83:617–21.
- [3] Benthem L, Keizer K, Wiegman CH, de Boer SF, Strubbe JH, Steffens AB, et al. Excess portal venous long-chain fatty acids induce syndrome X via HPA axis and sympathetic activation. *Am J Physiol* 2000;279:E1286–93.
- [4] Berger K, Winzell MS, Mei J, Erlanson-Albertsson C. Enterostatin and its target mechanisms during regulation of fat intake. *Physiol Behav* 2004;83:623–30.
- [5] Bray G, Lee M, Bray T. Weight gain of rats fed medium-chain triglycerides is less than rats fed long-chain triglycerides. *Int J Obes* 1980;4:27–32.
- [6] Cox JE, Kelm GR, Meller ST, Spraggins DS, Randich A. Truncal and hepatic vagotomy reduce suppression of feeding by jejunal lipid infusions. *Physiol Behav* 2004;81:29–36.
- [7] Cox JE, Kelm GR, Meller ST, Randich A. Suppression of food intake by GI fatty acid infusions: roles of celiac vagal afferents and cholecystokinin. *Physiol Behav* 2004;82:27–33.
- [8] Denbow DM, Van Krey HP, Lacy MP, Watkins BA. The effect of triacylglycerol chain length on food intake in domestic fowl. *Physiol Behav* 1992;51:1147–50.
- [9] Duval C, Augé N, Frisach MF, Casteilla L, Salvayre R, Nègre-Salvayre A. Mitochondrial oxidative stress is modulated by oleic acid via an epidermal growth factor receptor-dependent activation of glutathione peroxidase. *Biochem J* 2002;367:889–94.
- [10] Ferrari B, Arnold M, Carr RD, Langhans W, Pacini G, Bodvarsdottir TB, et al. Subdiaphragmatic vagal deafferentation affects body weight gain and glucose metabolism in obese male Zucker (fa/fa) rats. *Am J Physiol* 2005;289:R1027–34.
- [11] Friedman MI. Fuel partitioning and food intake. *Am J Clin Nutr* 1998;67:513S–8S.
- [12] Furuse M, Choi YH, Mabayo RT, Okumura JI. Feeding behavior in rats fed diets containing medium chain triglyceride. *Physiol Behav* 1992;52:815–7.
- [13] Grekin RJ, Vollmer AP, Sider RS. Pressor effects of portal venous oleate infusion. A proposed mechanism for obesity hypertension. *Hypertension* 1995;26:193–8.
- [14] Jambor de Sousa UL, Arnold M, Langhans W, Geary N, Leonhardt M. Caprylic acid infusion acts in the liver to decrease food intake in rats. *Physiol Behav* 2006;87:388–95.
- [15] Langhans W. Role of the liver in the control of glucose-lipid utilization and body weight. *Curr Opin Clin Nutr Metab Care* 2003;6:449–55.
- [16] Langhans W. Hepatic and intestinal handling of metabolites during feeding in rats. *Physiol Behav* 1991;49:1203–9.
- [17] Lavau M, Hashim S. Effect of medium chain triglyceride on lipogenesis and body fat in the rat. *J Nutr* 1978;108:613–20.
- [18] Leonhardt M, Langhans W. Fatty acid oxidation and control of food intake. *Physiol Behav* 2004;83:645–51.
- [19] Mélone J. Vagal receptors sensitive to lipids in the small intestine of the cat. *J Auton Nerv Syst* 1986;17:231–41.
- [20] Meyer JH, Hlinka M, Tabrizi Y, DiMaso N, Raybould HE. Chemical specificities and intestinal distributions of nutrient-driven satiety. *Am J Physiol* 1998;275:R1293–307.
- [21] Ramirez I, Tordoff MG, Friedman MI. Satiety from fat? Adverse effects of intestinal infusion of sodium oleate. *Am J Physiol* 1997;273:R1779–85.
- [22] Scheurink AJW, Steffens AB, Bouritius H, Dreteler GH, Bruntink R, Remie R, et al. Adrenal and sympathetic catecholamines in exercising rats. *Am J Physiol* 1989;256:R155–60.
- [23] St Onge MP, Jones PJ. Physiological effects of medium-chain triglycerides: potential agents in the prevention of obesity. *J Nutr* 2002;132:329–32.
- [24] Steffens AB. A method for frequent sampling of blood and continuous infusion of fluids in the rat without disturbing the animal. *Physiol Behav* 1969;4:833–6.
- [25] Strader AD, Woods SC. Gastrointestinal hormones and food intake. *Gastroenterology* 2005;128:175–91.
- [26] Stubbs R, Harbron C. Covert manipulation of the ratio of medium- to long-chain triglycerides in isoenergetically dense diets: effect on food intake in ad libitum feeding men. *Int J Obes* 1996;20:435–44.
- [27] Surina-Baumgartner DM, Langhans W, Geary N. Hepatic portal insulin antibody infusion increases, but insulin does not alter, spontaneous meal size in rats. *Am J Physiol* 1995;269:R978–82.
- [28] Randich A, Spraggins DS, Cox JE, Meller ST, Kelm GR. Jejunal or portal vein infusions of lipids increase hepatic vagal afferent activity. *NeuroReport* 2001;12:3101–5.
- [29] Tietze F. Enzymatic method for quantitative determination of nanogram amounts of total and oxidized glutathione: applications to mammalian blood and other tissues. *Ann Biochem* 1969;27:507–22.
- [30] Tso P, Liu M. Apolipoprotein A-IV, food intake, and obesity. *Physiol Behav* 2004;83:631–43.
- [31] Van Wymelbeke V, Himaya A, Louis-Sylvestre J, Fantino M. Influence of medium-chain and long-chain triacylglycerols on the control of food intake in men. *Am J Clin Nutr* 1998;68:226–34.
- [32] Van Wymelbeke V, Louis-Sylvestre J, Fantino M. Substrate oxidation and control of food intake in men after a fat-substitute meal compared with meals supplemented with an isoenergetic load of carbohydrate, long-chain triacylglycerols, or medium-chain triacylglycerols. *Am J Clin Nutr* 2001;74:620–30.
- [33] Williamson JR, Browning ET, Scholz R, Kreisberg RA, Fritz IB. Inhibition of fatty acid stimulation of gluconeogenesis by (+)-decanoylcarnitine in perfused rat liver. *Diabetes* 1968;17:194–208.
- [34] Yeh YY, Zee P. Relation of ketosis to metabolic changes induced by acute medium-chain triglyceride feeding in rats. *J Nutr* 1976;106:58–67.
- [35] Yox DP, Ritter RC. Capsaicin attenuates suppression of sham feeding induced by intestinal nutrients. *Am J Physiol* 1988;255:R569–74.
- [36] Yox DP, Brenner L, Ritter RC. CCK-receptor antagonists attenuate suppression of sham feeding by intestinal nutrients. *Am J Physiol* 1992;262:R554–61.